SOME OBSERVATIONS ON THE TRANSMISSION OF FILARIA BANCROFTI IN QUEENSLAND

by

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The adult *Filaria bancrofti* was discovered in Brisbane in 1876 by Dr. Joseph Bancroft, though its embryos had been known for thirteen years previously. Brisbane has a further historical connection with the disease, as the life history was actually worked out by Manson from mosquitoes infected in Brisbane and sent to him by Bancroft. The species was the common *Culex quinquefasciatus* Say *(C. fatigans, C. ciliaris)*. Bancroft confirmed Manson's results and also investigated others, but since that time no attempt has been made to find out if there are any other culprits among the Queensland mosquitoes, though Bancroft has worked on the carriers of *Dirofilaria immitis*.

Much work has been done in other parts of the world and complete development has been observed in the following (2):

1. *Culex quinquefasciatus* Say.
2. *Aedes (Stegomyia) variegatus* Doleschall (Syns. *Culex scutellaris* Walker nec Theobald, *Stegomyia pseudoscutellaris* Theobald).
   Bahr (1) has shown that in Fiji this is a more important intermediary than *C. quinquefasciatus*, which also occurs there.
3. *Aedes (Finlaya) tagoi* Theobald.
   Yamada (3) has shown that this is an efficient host in Japan.
4. *Taeniarhynchus (Mansonoides) africanus* Theobald.
   Either this or a closely allied and frequently associated species, *T. (M.) uniformis* Theobald, has been found to be a carrier in tropical Africa.
5. *Anopheles (Myzomyia) rossi* Giles (? *subpictus* Grassi).
   Found by James to be a carrier in India.
6. *Anopheles (Myzomyia) costalis* Theobald.
   Found by Annett, Dutton, and Elliott to be a carrier in West Africa.
7. *Anopheles algeriensis* Theobald is a host in Tunis.

In many other mosquitoes, partial development is known to occur but their role as host is not proven, as infective forms have not been found in the proboscis.

As the Hookworm Campaign is making a survey of Filaria in Queensland, interest has been aroused in the subject, and it was thought advisable to conduct investigations into the efficiency, as transmitters, of the different mosquitoes in

Brisbane. For this purpose, the four most abundant species, viz., *Culex quinquefasciatus* Say, *C. annulirostris* Skuse, *Aedes vigilax* Skuse, and *A. aegypti* L. (syn. *Stegomyia fasciata*), were selected as being of the greatest practical importance.

At first engorged mosquitoes were collected at random from different parts of Brisbane and, after being kept a few days to allow any parasites within them to develop, were dissected. Although many hundreds of individuals of the four mentioned species were thus examined, no parasite was seen, so that it is safe to assume that no large percentage of the general mosquito population is infected.

Method. To trace the exact cycle, mosquitoes of the four different species were infected by feeding on blood containing microfilaria. Only mosquitoes collected as larvae and bred in the laboratory were used. They were fed on bananas, dates, sugar and water, and were starved for thirty-six hours before their meal of infective blood, which was always their first, and (except in one instance) their last, meal of blood.

As soon as practicable after engorgement, the insects were transferred to a mosquito-net cage and examined at intervals. They were killed by chloroform and immediately dissected, and teased out in saline on a slide so that the developing filariae might be examined alive. The chief difficulty throughout was to obtain persons with sufficient microfilariae in their blood who were willing to let the mosquitoes bite them. Heavy infections with microfilariae are rare in Brisbane.

Experiments with CULEX QUINQUEFASCIATUS. The first successful experiment began on February 10, 1923, when specimens of *C. quinquefasciatus* were fed on man D., harbouring 40–60 microfilariae per 20 cmm. blood. Next day one was examined and fourteen microfilariae found in the stomach; thereafter daily examinations were made and progress noted. The general development observed agreed with that described by Bahr (1) for the development of the Fijian microfilaria in *Aedes variegatus (Stegomyia pseudoscutellaris)* and similar variations in the rate of development were noted (Fig. 5).

On February 16 (i.e., 6th day), sausage-shaped forms were found among the thoracic muscles. Two measured were 0·192 mm. × 0·032 mm. and 0·220 mm. × 0·032 mm. respectively. They showed the anterior and posterior V-spots (the anterior somewhat displaced by the multiplication of nuclei, as described by other authors), but the tapering at the end was much more abrupt than that of a similar form figured by Bahr (Plate vi, Fig. 3).

In one of the mosquitoes preserved and sectioned seven such developing forms were found in the thoracic muscles, but one had an opaque brown appearance, which suggested that chitinisation was already taking place.

On February 20 (i.e., 10th day) another mosquito showed six developing forms in the thorax. In size these ranged from 0·86 mm. × 0·016 mm. to 1·04 mm. × 0·018 mm. (Fig. 2), and showed complete alimentary canal with an

oesophageal bulb. Although no actual ingestion of muscle fibres, as reported by Bahr, was seen, in a later experiment a worm at about this stage showed dark, solid material in the anterior part of the intestine (Fig. 5).

On February 22 (i.e., 12th day) a mosquito appeared sluggish and sick It was dissected and found to contain nineteen completely or almost completely developed filariae (Fig. 3); there were two in the proboscis, two in the head and neck, eight among the thoracic muscles, and seven in the abdomen. Thus in this experiment the development was complete and the mosquito became infective after 11½ days. In mosquitoes preserved and sectioned, at least as many larvae were found, some occurring also in the legs and antennae (Fig. 4), while all the insects showed proboscid infection.

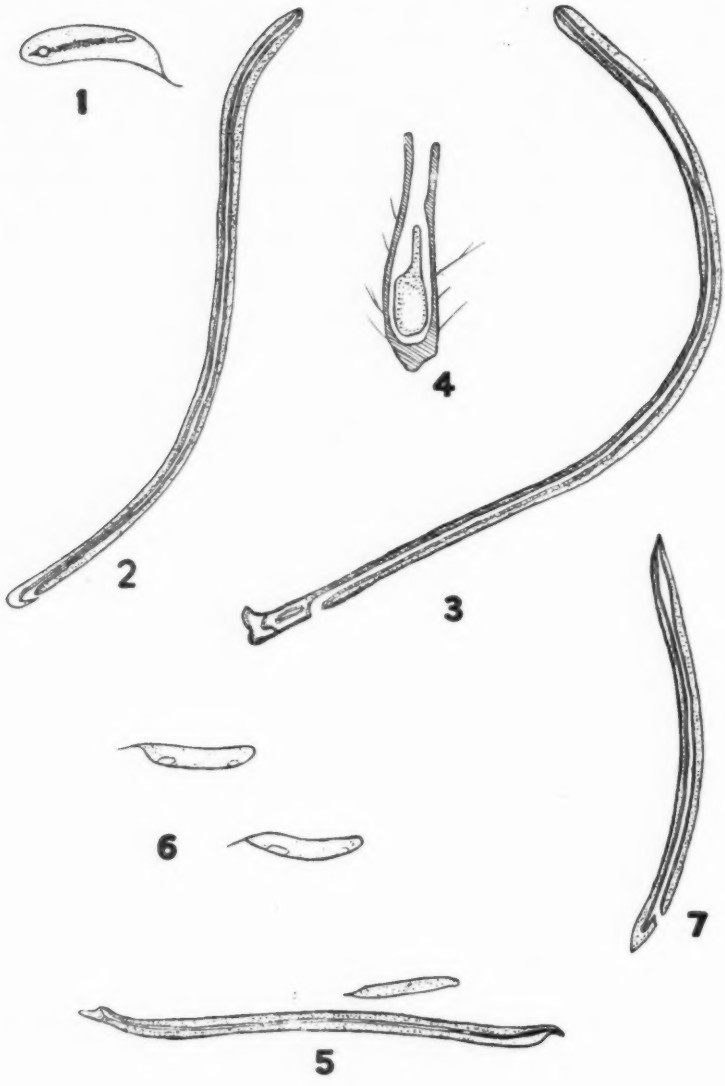
As *C. quinquefasciatus* proved to be such an efficient host, it was always used as a control in subsequent experiments. During the summer months in Brisbane full development was found to take from 9½ to 16 days. The former very short time occurred in January, 1924, in excessively hot and humid weather; mosquitoes were fed on January 7 and were infective on January 17. As I left for Mackay on January 15, the mosquitoes spent a day and a half at sea, but except that they were in the cabin of a ship (which might occur naturally) conditions remained the same.

There was some mortality, though not a large one, among the *C. quinquefasciatus*; sometimes mosquitoes appeared sluggish and sick for a few days and then recovered. This was probably due to the escape of the larval filariae, which relieved the mosquito, as such insects were found to be filariae-free or lightly infected (though belonging to an infected brood), the infection where it occurred being proboscid. In none of these older mosquitoes were larval filariae ever found in the abdomen. It was thought that possibly they had escaped with the eggs. The water in which these were laid was therefore centrifuged and examined, but none were found, though other Nematodes (Anguillulidae) were present. It is more probable that the filariae had escaped into the fruit on which the insects were fed (those from abdomen and legs moving forward into the proboscis as each successive pair escaped), though Manson, Low, and Vincent found that this does not occur with *D. immitis*.

In warmed saline, the larvae were often seen escaping from the extreme tip of the proboscis between the labellae, but went straight out, anterior end first, and not in loops, as shown by Bahr (1) (Photograph iv).

An attempt was made to trace the course of the larval filariae through the skin, but the infected mosquitoes could not be induced to bite a shaven guinea pig.

Experiments with CULEX ANNULIROSTRIS. On March 28, 1923, *C. annulirostris* and *C. quinquefasciatus* were fed on man H., harbouring 70–90 microfilariae per 20 cmm. of blood. Owing to accidents, only four engorged mosquitoes were successfully transferred to the cage. Individuals were dissected after 6, 15, and 22 days, but no traces of developing larvae were found. On April 24,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however (i.e., after 27 days), two sausage-shaped forms were found in the thoracic muscles. These were 0·168 mm. × 0·020 mm. and 0·184 mm. × 0·024 mm. (Fig. 6). In *C. quinquefasciatus*, infected at the same time, the worms reached the corresponding stage on the fourth or fifth day. However, these worms in *C. annulirostris* showed no evidence of degeneration and would probably have developed further.

In the second experiment, *C. annulirostris* were fed on man H., showing only three microfilariae per 20 cmm. of blood, on February 22, 1924. Only five engorged insects were obtained, and owing to the very small filaria-content of the blood it is unlikely that all were infected. They were examined at intervals, but nothing was found until the last was searched on March 12 (i.e., 18½ days), when one developing form, 0·64 mm. × 0·024 mm. was seen. It had a complete alimentary canal with an oesophageal bulb, and would probably have attained complete development.

These experiments with *C. annulirostris* are not at all conclusive owing to the small numbers of mosquitoes engorging and the light infection in the last case. It is likely that further experiments will show that *C. annulirostris* may act as a host under favourable conditions, though the development of the worms is slow

Experiments with AEDES VIGILAX. Two attempts were made to follow the development in this insect. Mosquitoes were fed on March 30 on man H., harbouring 30–50 microfilariae per 20 cmm. of blood, and dissection of engorged mosquitoes showed large numbers in the stomach.

On the third day (April 2) two *A. vigilax* were dead, and thereafter great mortality occurred until by the seventh day (April 6) all were dead. Uninfected *A. vigilax* of the same brood lived about three weeks longer. The control, *C. quinquefasciatus*, all lived and became infective on the sixteenth day (April 15).

In November another attempt was made. On the night of November 12 two batches of *A. vigilax* and control, *C. quinquefasciatus*, were fed on two

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REFERENCE TO FIGURES.

Camera lucida drawings to show development of *F. bancrofti* in *C. quinquefasciatus* and *C. annulirostris*. 1, 2, 5, 6, 7 from living specimens. 3 and 4 were fixed and stained with haematoxylin.

Fig. 1. 6th day in *C. quinquefasciatus* (Feb. 10th–16th). Size, 0·192 mm. × 0·032 mm.
Fig. 2. 10th day in *C. quinquefasciatus* (Feb. 10th–16th). Size, 1·04 mm. × 0·018 mm.
Fig. 3. 12th day in *C. quinquefasciatus* (Feb. 10th–22nd). Infective form from abdomen showing oesophageal bulb, post-anal extension of the gut, terminal papillae, etc.
Fig. 4. Section of antenna showing infective form of filaria within it.
Fig. 5. Larvae from thoracic muscles of *C. quinquefasciatus* after 11½ days (Nov. 12th–24th), showing variation in size and also solid matter in intestinal canal.
Fig. 6. Two "sausage-shaped" forms found among thoracic muscles of *C. annulirostris*.
Fig. 7. Larva from thoracic muscles of *C. annulirostris* after 18½ days. Size, 0·64 mm. × 0·024 mm.

patients, Dora and Rita, both showing about 10–20 microfilariae per 20 cmm. of blood. Here again mortality occurred from the second day, in spite of all attempts to prolong life by changing the diet and by feeding on human blood. Up to the seventh day no trace of microfilariae could be found on dissection, but in one *A. vigilax*, which died on the sixth day and which was sectioned, a line of nuclei seen between the muscle fibres may possibly have denoted a degenerating larva.

On November 21, the last *A. vigilax* died and on dissection one tiny worm was found in the thorax; it was 0·09 mm. × 0·005 mm., and of the typical sausage form, rounded at one end, pointed at the other. The anterior V-spot was indistinguishable, but there was a trace of the posterior V-spot.

In the two experiments, this was the only worm found which showed any trace of development, and though infection had occurred 8½ days previously, it was at a stage reached by the larvae in *C. quinquefasciatus* during the second day. At the same time the worms in the control, *C. quinquefasciatus*, were 0·29 mm. × 0·031 mm., and their development was complete in fifteen days.

A. vigilax is therefore not an efficient host, though partial development may occur; and, moreover, the microfilariae seem to be lethal to it.

Experiments with AEDES AEGYPTI. As this mosquito only bites by day when microfilariae are absent from peripheral blood it was difficult to infect it. Attempts were made to infect mosquitoes by feeding on a patient, Q., whose microfilariae showed a reversed periodicity as he was on night duty. Few mosquitoes fed, and nothing was found in any subsequently killed and examined up to the twenty-fourth day, but as the microfilariae were not very abundant in the blood all may not have been infected. As *C. quinquefasciatus* will not bite by day, it could not be used as a control.

In the second experiment, the mosquitoes fed between 6.30 p.m. and 7 p.m. on January 7 on a man H., whose blood at that hour showed from 10–20 microfilariae per 20 cmm. of blood. Many of the insects bit and were successfully transferred to the cage. Two which were examined were found to harbour three and five microfilariae respectively in the stomach.

These mosquitoes were taken to Mackay on January 15, but did not show any ill effects from the journey, and no mosquito died throughout the experiment. Mosquitoes were examined at intervals up to January 30 (22½ days infected), when I left Mackay, but no trace of any developing worm was ever found, though many were dissected and also sectioned. The worms in the control, *C. quinquefasciatus*, on the other hand, evidenced remarkably quick development, the mosquitoes becoming infective in 9½ days.

A. aegypti, then, appears to be entirely inhospitable to *F. bancrofti* in Queensland.

Adult Filaria bancrofti. No investigations were made on the adult, as this is thoroughly known. In a section of a lymphatic gland, with portions of a male

and female filaria in it, a large amount of fibrosis had taken place. It is noteworthy that no uncoiled larvae were to be seen within the worm (all were surrounded by the chorional envelope) and eggs occurred in the gland spaces. These facts seem to support Manson's suggestion that the blocking of the lymph channels may be due to prematurely extruded ova; perhaps the oviparous and viviparous states may alternate.

SUMMARY.

1. *C. quinquefasciatus* is a very efficient host for *F. bancrofti* in Queensland. Mosquitoes were found to be infective in very hot weather 9½ days after feeding on an infected patient, but the usual summer time for complete development is from 11–16 days.
2. In *C. annulirostris* partial development was noted, and under very favourable circumstances complete development may occur.
3. *A. vigilax* is not an efficient host and the microfilariae are injurious to it. Partial but very slow development may occur.
4. In *A. aegypti* no development at all occurs.

My thanks are due to Dr. W. Sweet, Director of the Hookworm Campaign, Brisbane, for permission to do some of the work in the laboratory and with the apparatus of the Hookworm Campaign; to Professor E. Goddard, for the loan of apparatus; and to Mr. L. E. Cooling, of the Australian Institute of Tropical Medicine, for assistance in naming the mosquitoes.

NOTE.

Since writing the above, Dr. T. L. Bancroft (Eidsvold) has informed me that he has seen proboscid infection in *Culex annulirostris*, so that this form should be added to Edwards' list. Dr. Bancroft, however, does not separate *C. sitiens* Wiedemann from *C. annulirostris* Skuse, and the host he saw may have been the salt-water breeder, which I did not test.

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THE EFFECT OF DOING WORK, ON THE HEAT PRODUCTION OF MUSCLES DURING ACTIVITY

by

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A long series of researches, from the early work of du Bois Reymond to that of Fletcher and Hopkins, of A. V. Hill and of Meyerhof in recent years, has made it very clear to us that muscular contraction is the result of the liberation of lactic acid from carbohydrate during excitation. It follows, therefore, from purely theoretical considerations, that the heat liberated by a muscle in undergoing shortening during a single twitch must be composed of two parts: there is the heat which is the concomitant of the chemical reactions, which produce the surface tension changes within the sarcomeres; and there is the heat which represents the kinetic energy dissipated during the process of change of shape that the muscle undergoes.

The second of these quantities must be affected when the contracting muscle lifts a weight. But whether the first part can be affected under the same conditions cannot be decided from first principles. It is possible that the increased tension to which the fibres are subjected when a muscle is doing work may change the irritability of the excitable membranes of Krause. The fact, however, that the strain is borne by the fibrils and not by these membranes would indicate that this is not so, and I shall try to show, below, that the observed excess of heat produced by muscles doing work over that produced when shortening but not doing work, is to be explained entirely on the basis of the dissipation of energy due to the movements of fluids within the sarcomeres that the microscope reveals, and that it is not necessary to invoke any such changes of irritability.

It is to the early work of Heidenhain, but especially to the later work of Fick, that accurate data on this question are due. Fick observed that a muscle shortening under a tension often liberated considerably more heat than one contracting isometrically, and drew the conclusion that the actual doing of work was able to bring about an expenditure of chemical energy (2). This conclusion, discarded for many years mainly through Blix's interpretation of the phenomenon in terms of increased length of fibres, has recently been revived by W. O. Fenn (1).

Employing the highly sensitive myothermal technique of A. V. Hill, he shows that during the performance of muscular work there is an excess heat output above the isometric value, that the excess is roughly proportional to the work done, and that it passes through a maximum with increase of weights lifted, as does the work also. He concludes from this that "the energy liberated by the contraction of a single muscle fibre for a given stimulus is not dependent solely upon the initial mechanical and physiological condition of the muscle, but can be modified by the nature of the load which the muscle discovers it must lift after the stimulus is over."

Our scanty knowledge of the physical nature of irritability does not as yet enable us definitely to deny this conception of Fick and of Fenn that increased tension causes increased chemical activity within the muscle (evidently through acting on the two excitable membranes). At the same time, it must be admitted that the theory of the intimate nature of muscle action as entertained by many physiologists to-day, viz.: that it is due to a change in the shape of colloidal complexes within the fibres, from an ovoid to a more spherical shape, due to an increased surface tension of these complexes, cannot account for their observations without resorting to some such hypothesis as that suggested by Fenn. But when we consider that muscular contraction is due to extensive movements of fluids through capillary tubes within the sarcomeres (reversal of striations) (6), and that these movements must be affected by the application of tension to the whole muscle, then the cause of this extra heat production at once becomes apparent.

The contractile machine of muscles, as I have been able to infer from microscopic observations, consists (in each sarcomere) of a number of minute collapsible sacs, each communicating by a capillary tube (usually ultramicroscopic in size) with a porous sponge-work in the middle of the sarcomere (6). Contraction consists simply in a withdrawal of fluid from the porous sponge-work through the capillary tubes into the sacs, which therefore dilate; this causes swelling of the bases of the sarcomeres, which therefore shorten. At relaxation the fluid again passes through the tubes and re-enters the porous network. The movements take place through the changes of interfacial tension between the fluid and the walls of the tubes, under the influence of the electrostatic field produced by the electrical double layer on the surface of the lactic acid as it diffuses past the walls of the sarcomeres.

In moving through these capillaries a considerable part of the kinetic energy of the fluid must disappear as heat. According to the law of Poiseuille for the flow of viscous fluid through a capillary tube, the time of efflux of a given volume of liquid is proportional to the length of the tube, inversely to the difference of pressure at the two ends, and inversely as the fourth power of the diameter of the tube. If, therefore, a muscle is allowed to shorten after stimulation, then we may write:

$$H = I + k\left(\frac{l \times 1 \times q \times v}{r^4}\right)$$

where H is the total heat liberated by a muscle in contracting (but not relaxing) in response to a single shock, I is the isometric heat, l the length of the tubes, r their radius, and q the quantity of fluid that has moved through the tubes with a velocity v; k is the constant which expresses the dissipated kinetic energy in terms of l, r, q, and v.

If such a muscle is lifting a weight while contracting, l will be increased roughly, but not accurately, in proportion to increase of weight (muscles exhibit the phenomena of afterloading markedly) (3). The area of cross section of the capillary tubes will therefore decrease roughly in the same proportion. With increase of weight, the second factor in Poiseuille's equation will decrease, for, while the force driving the fluid through the tubes (decrease of surface tension) remains the same, the resistance of the collapsed sacs at the other end of the tubes to filling, increases in direct proportion to the weight: i.e., q, or its integral, S, the shortening of the whole muscle, varies inversely as the weight lifted. Lastly v, the average velocity of the fluid along the tubes due to the acceleration imparted to it by the surface tension force will vary inversely as the weight lifted. Expressing the factors in the above equation as proportional to the weight lifted (W), or the degree of shortening S, we may write:

$$H = I + K\left(\frac{W \times W^2 \times S \times 1}{W}\right)$$

$$= I + K(W^2 S)$$

This would give the excess heat in proportion to work done if the change in the values of l and r could remain inappreciable during the shortening. This, however, is not the case, and the factor K (W^2 S) will approach much nearer to K (WS). The actual calculation cannot be made; it must be one of extreme complexity, and will depend upon the velocity of the moving fluid at any moment through tubes of varying diameter. The velocity, in its turn, will depend on such factors as the rate of liberation of lactic acid and on the relative velocity of its ions. The above equation suffices to show that the excess heat liberated must depend both on the magnitude of the weight lifted (W) and the distance through which it is lifted (S); that it will be zero for very large weights (isometric contraction) and will increase in value as the weight is decreased, and then, passing through a maximum, will again decrease as the weight decreases, again reaching a minimum as the weight becomes zero. In this last case the excess heat will be determined by the weight of the muscle alone.* The weight pro-

* Hill was unable to detect any difference in the heat output of a muscle contracting isometrically and one released after the maximum tension had developed (4). This merely means, of course, that the very small quantity of heat which accompanies the actual shortening is within the limits of the experimental error.

ducing a maximum excess heat will be in the neighbourhood, but may not necessarily be identical with that which gives the maximum amount of work.

The magnitude of this excess heat may be estimated from some observations of Hartree and Hill (3) and Fenn (1). Hartree and Hill found that stretching a resting muscle caused a minute rise in temperature; when the muscle was again relaxed the temperature first fell, but then rose again. Two thermal changes are evidently involved, one reversible, the other irreversible. The irreversible change is due evidently to frictional loss corresponding to a slight movement of fluids within the tubes. The reversible heat change would appear to arise in the following manner. Tension on the muscle must cause lateral compression of the cylindrical sarcomeres. The porous network will be pressed together, and the free surface of its fluid contents will decrease.* Kelvin has applied the principles of thermodynamics in such a case to show that when the free surface area of a fluid is decreased its temperature must rise (5).

Fenn has observed that when a stretched resting muscle is released and then again stretched (without being stimulated) the heat developed ("viscous loss") due to this slight movement of fluid is as much as 2 per cent. of the total heat developed by a muscle on contracting. Hartree and Hill (3), applying very heavy weights to the frog's sartorius, obtained a heat output which may be as much as 20 to 50 per cent. that of a strong twitch. When, therefore, a muscle is actively doing work the extensive fluid movements that are going on are quite able to account for the excess of heat liberated, and it is not necessary to seek a chemical origin for it.

The above considerations of excess heat output with performance of work have been concerned only with the excess during the production of the twitch. It is clear, however, that they apply also to the relaxation of the muscle. In a simple twitch the rate of relaxation is considerably slower than the rate of contraction. From the law of Poiseuille it therefore follows that the energy dissipated during the relaxation period is in proportion less than that wasted during contraction. The measurements of Fenn show that such a difference can really be detected; his figures indeed are surprisingly high. While the excess heat for a whole twitch may be 1·8 times the maximum work, he finds the excess heat for

* The validity of this statement depends on the following considerations: The free area of the porous network must be regarded as greatly exceeding that of the capillary tubes and the collapsible sacs. During contraction the movable fluid retreats from the porous network, which *must* become filled with water vapour. At relaxation the tension between the fluid and its vapour again increases, and (provided the angle of contact of the fluid with the tubes be acute) the increased tension will draw the fluid into the porous network again, in the same way as a fluid is drawn up a capillary tube by the tension between the fluid and the vapour above it. For the continued working of this surface tension machine it is necessary that the vapour should not all condense at relaxation. Frequently (especially in insect flying muscles) a clear space can actually be seen in the middle of the porous material ("Hensen's Line"). Now a lateral compression of this porous network will not cause any change in the area of contact of the vapour and the liquid, but it *will* produce a decrease of the area of contact between the fluid and the walls of the sarcomere, and it is to this decrease that the temperature changes are due.

contraction to be as much as 1·3 times this work, i.e., nearly three times the excess heat of relaxation.

The excess heat accompanying the performance of work is to be looked upon as arising through the dissipation of the kinetic energy of the fluids whose movements produce the contraction, and not as having a chemical origin. From this it will follow that there will be no relation between the work performed and the quantity of lactic acid liberated (or oxygen absorbed and carbon dioxide liberated during the recovery period). It is true that during the performance of work an excess of chemical energy will be liberated as heat, but this arises in a different way, namely, through the failure of the valvular mechanism to exert its full effects when the fibres are prevented from undergoing a maximum shortening. It is not justifiable, therefore, as Fenn has done, to draw a comparison between the increased oxygen consumption of a dilated heart and the increased output of skeletal muscles doing work. In the case of the heart the increased oxygen intake is to be correlated with the increased chemical activity due to the opening of the valvular mechanism as the fibres are stretched. A stretched skeletal muscle would show the same increased oxygen intake and corresponding heat liberation, but it is the excess heat liberation *above the isometric* value that Fenn has measured, and this has nothing but a physical origin.

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THE STEROL OF BOLETUS GRANULATUS

by

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The definite accelerative influence exerted by cholesterol on the rate of growth and metastasis-formation of experimental Flexner–Jobling carcinoma (8), and the fact that cholesterol emulsions will greatly increase the rate of reproduction of isolated infusoria (1), render evident its catalytic effect on the proliferation rate of certain types of cells.

The remarkably rapid growth of the fruit of certain fungi suggests that the study of the sterols present in this group might throw some light on their biological activity. With this in view, a study of the sterols in *Boletus granulatus* was undertaken. The fruit of *Boletus granulatus*, under favourable conditions, grows ten inches in diameter, or more, within seven days.

The fruit was collected, and dried in a current of warm air (40°C.). It was then reduced to powder and extracted with acetone in a continuous extraction apparatus. The extract was then distilled free from acetone, and the resulting tarry liquid allowed to stand for several days. During this time a few crystals made their appearance. The separation of these from the tarry mass was not easily accomplished. However, a partial separation was effected by the use of light petroleum, in which the crystals were soluble only with difficulty.

The crude product, after washing with petrol, was recrystallized several times from alcohol.

From every 10 kilos of fresh fungus about 50 mgms. of long flat crystals were obtained. The crystals were soluble in most organic solvents, and melted with darkening at 161°C.

The addition of sulphuric acid to a chloroform solution of this substance gave a red colouration at the junction of the liquids (Liebermann-Burchart Reaction). The sterol was identical in crystalline form and melting-point with that isolated by Ikeguchi (2) from several allied species of fungi. His analysis of the sterol, to which he gave the name Mycosterol, indicates an empirical formula of $C_{30}H_{48}O_2$. It forms, like cholesterol, a mon-acetyl derivative, indicating one hydroxyl group, but, unlike cholesterol, it forms no addition product with bromine, showing the absence of unsaturated carbon atoms. On oxidation with chromic acid, Ikeguchi (2) obtained from Mycosterol a trihydroxy sterol.

Consideration of the above facts suggests that the sterol contains one atom of oxygen bound up in the molecule as an hydroxyl group, the other atom being bound in some other form. It contains no — CH = CH—groups.

A cholesterol derivative answering to this description has been prepared by Westphalen (9) by oxidation of cholesterol with Benzoyl peroxide, the unsaturated linkage becoming oxidized under these conditions.

A sample of the α cholesteryl oxide (Westphalen's product) was supplied to me by Professor Robertson. The crystals, α cholesteryl oxide, melted at 140°C. On hydrolysis by superheated steam a trihydroxy sterol resulted, with a melting-point of 240°C., this substance being identical with the dehydrocholestantriol of Pickard and Yates (6).

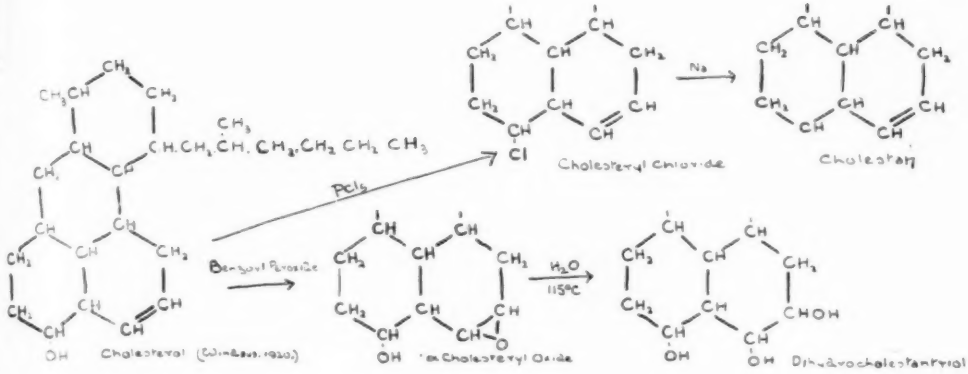
The properties of α cholesteryl oxide, however, are not similar to those of the sterol from the fungus. The difference is clearly demonstrable by its action towards arsenic trichloride (Kahlenberg's Test). The sterol of *Boletus granulatus*, if dissolved in pure anhydrous arsenic trichloride, will immediately produce a violet colouration.

Cholesterol, under similar conditions, will give a red reaction (3), whereas α cholesteryl oxide dissolves giving no colour whatever.

With the view of determining the actual chemical groupings responsible for the colouration, several sterols and sterol derivatives were tried. The results are tabulated below:

Sterol.	Preparation.	Colour in $AsCl_3$
Cholesterol	From Brain Tissue	Red
α cholesteryl oxide	Mild oxidation of cholesterol	Nil
Dihydrocholestantriol	Hydrolysis of α cholesteryl oxide	Nil
Cholesteryl acetate	Acetic anhydride on cholesterol	Red
Cholesteryl chloride	Phosphorous pentachloride on cholesterol	Red
Cholestan (Hydrocholesterylen) (5)	Sodium amalgam on cholesteryl chloride	Red
Oxycholesterol	Oxidation of cholesterol	Blue
Phytosterol	From maize embryos	Nil
Mycosterol	From *Boletus granulatus*	Violet

The above table shows that destruction of the — CH = CH — group in cholesterol will at the same time destroy its power to produce a red colour with arsenic trichloride. Moreover, the fact that destruction of the OH group has no such effect is shown by the positive reaction with cholestan and cholesteryl chloride. This would suggest that the production of colour is conditional on the integrity of the unsaturated linkage. However, Phytosterol, which is considered



by Windaus (10) to be an isomer of cholesterol, and which contains both an unsaturated linkage and an OH group, will give no colouration with the reagent.

The oxycholesterol of Lifschütz (4) gives a blue colouration. This substance was shown by him to be a dihydroxy sterol derivative.

The biological action of the sterol from *Boletus granulatus* will be discussed in a later paper.

I wish to thank Professor T. Brailsford Robertson for his kindness in supplying to me several of the cholesterol derivatives used in this work.

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THE SYMPTOMATOLOGY OF COMPLETE TRANSVERSE LESIONS OF THE SPINAL CORD: AN EXPERIMENTAL STUDY

by

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I. INTRODUCTORY.

In the first stage following complete transverse lesions of the spinal cord "not only is there complete motor and sensory paralysis in the region of the body below the level of the lesion, but there is also an absence of all reflex activity in the same region, no knee jerks nor ankle jerks, no abdominal, cremasteric nor plantar reflexes, and there is retention of urine and faeces." (Rennie (12)). This is the state of so-called "spinal shock" which von Monakow (10) describes as being due to a "diaschizis" taking place between the conducting cells. Sherrington (16) believes that this condition "is neither due to irritation by trauma, nor in the main a phenomenon of inhibition. The rupture of certain aborally conducting paths appears to induce it" (p. 246). Like von Monakow, he believes the condition to be due to a defect in transmission at the synapse following the rupture. He has recently stated (15) that this condition, as understood by the physiologist, is primarily nervous in origin and independent of vascular changes. The fall of arterial pressure accompanying spinal transection in front of the second thoracic level, he points out, is not the cause of the shock, for the head does not participate in it, although participating in the lowering of the blood pressure; also the shock following lower thoracic transection is approximately as severe as cervical transection, and section of both splanchnics does not cause shock.

Bing (2), however, inclines to the opinion that the most important factor in producing spinal shock is disturbance of the lymph and blood circulations which accompanies a total transverse lesion of the cord (p. 36). A similar view was taken by several members of the Medical School Staff at the University of Sydney, notably Professors J. T. Wilson and A. E. Mills. The attention of one of us (J.I.H.) was specially directed to the problem by the report of the variable duration of the first stage of flaccidity of the limb muscles in

complete transverse lesions described by Riddoch (13). Rennie (12) cites a case in which the tendon jerks and superficial reflexes did not reappear for five or six months, and he goes on to point out that "it must be recognized that this stage of absent reflexes may be indefinitely prolonged if muscular dystrophy sets in early" (p. 186). The general acceptance of Bastian's view (1) that permanent flaccidity followed this lesion has been due to the presence of such cases. "Why is it that in some cases this condition persists and goes on to complete atrophy with no return of any reflex activity, while in other cases the flaccidity gives place to rigidity and return of reflexes?" (12, p. 186). The experiments discussed in this paper were undertaken to attempt to give an answer to this question. Commencing in October, 1920, these experiments were devised and executed by us in collaboration, and one of us (J.I.H.) is responsible for the theoretical views expressed in the discussion of the results. Surgical comments will form the subject matter of a later paper elsewhere by the junior author.

The question arises, in the first instance, as to which is the variable factor responsible for this variable duration of diminished reflex excitability. Cases can only be included for comparison in which severe sepsis is excluded, for Head and Riddoch (5) have shown how profound may be the effect of this factor in diminishing reflex activity of the caudal segment. "The distal end, separated to a greater or less extent from the influence of the higher centres, is peculiarly susceptible to the divitalizing influence of long-continued toxaemia" (p. 482). The next possible variable factor is that of the degree of trauma causing the lesion. It is known from the work of Sherrington (16) that the degree of trauma is not the causal factor in the production of spinal shock. In his experiments, the division of the distal end of the spinal cord in a spinal animal in which the first stage of flaccidity had been replaced by the second stage of spasticity did not lead to a reappearance of shock. The second transection causes, for instance, only a slight and brief depression of the flexion reflex (16, p. 244). Moreover, he points out that unilateral section of the spinal cord leads to a unilateral and not a bilateral absence or depression of the reflexes, so proving that this injury does not produce a state of general shock. Also Pike (11) has shown that after freezing the spinal cord with ethyl chloride the reflexes of the skeletal muscles fail as completely as after anatomical transection.

It seemed to us that the variable factor which may in consequence determine the variability of the severity and the period of duration of spinal shock in transection of the spinal cord, in a given species, was the vascular supply of the caudal segment. It must be borne in mind that the blood supply of the spinal cord is both longitudinal and segmental, and naturally these two sources of supply are complementary to one another. The size and importance of the neural branches of the spinal arteries below any particular level of the spinal

cord will be determined by the amount of blood reaching the caudal segment by the longitudinal vascular network. The contribution which passes cranio-caudally consists of the supply contributed by the spinal branches of the vertebral arteries and the collaterals which have entered the medulla spinalis at various segments above the site under consideration. These considerations show that a complete transection of the spinal cord consists not only in the interruption of the longitudinal nervous pathways, but also in the division of the surrounding pial sheath and the vascular network in connection with it, with a consequent disturbance of the vascular supply of the caudal segment of the cord. The experiments recorded here were devised as an attempt to dissociate the effects of the nervous and vascular injury respectively from one another. Already the disturbances of vesical and rectal functions have been discussed in another connection (7). No further allusion to these symptoms will be made here.

The general results throw light upon two lines of enquiry, viz.: the causation of "spinal shock," and the factors responsible for its variable duration.

II. THE CAUSAL FACTORS IN THE PRODUCTION OF SPINAL SHOCK.

When the experiments were commenced, we thought it was possible that the vascular injury formed an important factor in the production of spinal shock. For instance, it seemed to us that it may provide an adequate explanation for the phenomenon described by Sherrington (16, p. 241), that the degree of spinal shock is, after high cervical transection, diminished proximo-distally, so that the upper extremities are affected to a greater extent than the lower extremities; for on the vascular hypothesis it may be supposed that the vascular disturbance would become relatively less marked in a cranio-caudally direction from the site of the lesion, on account of increased reliance distally upon the segmental arteries. Such a view proves to be erroneous.

The first experiment adopted to dissociate the effects of vascular and nervous disturbance was to divide the spinal medulla without injuring the larger vessels supplying the cord. A sub-pial resection was performed as follows:

Under open ether the spinal cord of a specimen of *Macacus rheusus* (I in the series) was exposed in the upper thoracic region. A small incision with a Graefe knife was then made into a relatively avascular part of the postero-lateral part of the exposed pia mater. A strabismus hook was directed into the medulla spinalis and passed round it within the pial sheath. The cord was easily broken through and partly withdrawn through the pial incision. Care was taken to leave no part of the spinal cord undivided, so that there was now present an empty pial bridge containing the large spinal vessels, which could still be seen intact and apparently viable.

The spinal shock which followed was profound. A minute after dividing the cord the strongest mechanical stimulus was incapable of producing a reflex contraction of the lower limb musculature, and no adequate stimulus could be found This depression of reflex activity included complete loss of vesical and rectal functions, although the anal sphincter was contracted. Two days later overflow incontinence of urine appeared. On the third day the bowels opened. An adductor flexion reflex of the left great toe was elicited on the application of nocuous stimuli to the sole. The left lower extremity showed some sign of return of tone. The right lower extremity responded similarly a day later. The knee jerks were not elicited until the eleventh day following the operation. A definite state of spasticity was present on the seventeenth day, the knee jerks being exaggerated; the flexion and crossed extensor reflexes could be elicited by faradaic stimulation, and reflex movements of the tail occurred on stimulating the perineum. Sherrington has pointed out that the shock is more profound in the monkey than in other laboratory animals, and the period of duration of the shock in this case was not extreme. Therefore, the experiment was completed by removing the vascular pial bridge in order to determine whether the removal of the longitudinal blood supply would destroy the activity of the caudal segment of the cord.

On the twenty-first day after the operation, when the signs of spastic paraplegia were well marked, the second operation was performed on this animal. On opening up the vertebral canal and exposing the spinal vessels in the intact pia mater, they were found to be apparently healthy. Only a thin white circular line of demarcation, which was at first difficult to locate, indicated the site of the sub-pial resection. A portion of the cord (1 cm. in extent), which included the divided zone, was now completely excised. A transient depression of the reflex activity of the distal segment followed this procedure, but fifteen minutes later the right knee jerk appeared, and soon afterwards the adductor reflex of the hallux was elicited. The spasticity was again marked two hours after the operation. Similar results followed such a procedure in goats. In goat 4 the pial bridge was severed on the fifth day. In five minutes the knee jerk was active. The flexion reflex was at first difficult to elicit, but in twenty minutes was actively re-established. On the vascular hypothesis it is possible that such an interval as five days will lead to some establishment of independence of blood supply to the caudal segment, so that the interval of time was reduced in subsequent experiments. After an interval of two hours forty minutes (goat 5) between the two operations, the knee jerk was active in seven minutes. After an interval of ten minutes (goat 6) the knee jerk was active at the end of five minutes. In a dog (dog 1) only an interval of two minutes was allowed. The knee jerks were elicited in two minutes, and were active at the end of ten minutes. Though not so valuable as the observations in the case of monkeys, in which spinal shock is

profound, these results support the evidence of the first experiment. These experiments show that "spinal shock" followed sub-pial resection, and that a subsequent division of the pia mater, when the stage of spasticity had been established, produced but a transitory effect of little importance. These facts point to the nervous disturbance as being the important element in the production of "spinal shock."

However, it is obvious that sub-pial resection of the cord entails considerable disturbance of the intramedullary circulation, and it follows that complete dissociation of the injury of the nervous and vascular elements was not obtained in this experiment. In a second experiment (*Macacus rhesus* 2), in order to interrupt the impulses descending from the higher centres with a minimum of surgical interference, the method adopted was to divide the cord physiologically by intra-pial injection of normal saline solution and of alcohol respectively.

The spinal medulla was first exposed in the mid-thoracic region of the spinal cord. The left motor area of the cerebral cortex was then exposed and the upper and lower extremity areas were localized by electrical stimulation. After determining the stimulus required to induce a reflex response of the lower extremity, ·35 cc. of normal saline was injected into the pial sheath of the exposed spinal cord. Immediate stimulation of the limb centres of the *cortex cerebri* revealed that the cord was physiologically transected between the upper and lower limb centres. It was found that reflex response could not now be elicited from the lower extremities. After four minutes from the time of the introduction of the saline block the reflexes of the lower extremities reappeared. The cortex was immediately stimulated, and a response was obtained in the lower as well as in the upper extremities, showing that the cord was no longer physiologically divided. This test indicated that the saline had been absorbed and the intra-pial pressure reduced, so that the reflex centres of the spinal cord were again under the influence of aborally directed impulses. It is significant that simultaneously with the recovery of the conductivity of the spinal cord the reflex activity of the spinal cord reasserted itself. Similarly, a permanent block was brought about by an injection of ·15 cc. of absolute alcohol (Macaque 2 and Macaque 3), and an immediate and persistent depression of the spinal reflexes below the site of the lesion was the result.

These experiments, in our opinion, rule out the idea that circulatory disturbance plays an important part in the causation of spinal shock. Of course, slight disturbances to the longitudinal vascular channels may have occurred in the course of intra-pial injection, both by compression and injury. However, the rapid reappearance of the reflex activity of the caudal portion of spinal cord shows that no permanent injury was inflicted upon this region of the cord.

This result is in accord with the effects of sudden rupture of long conducting paths in situations where no injury can occur to spinal vessels, as, for instance, in internal capsule lesions. Flaccidity is the immediate result of such lesions; the spastic condition is a later result.

In a penetrating analysis, Pike (11) has discussed the rôle played by rupture of the long conducting pathways in the production of spinal shock. He points out that the two possible interpretations of the reason for this effect are: (1) that the conductivity of the spinal reflex arc is maintained by impulses descending from higher levels; and (2) that morphologically old pathways from which the function has been moved to higher levels, regain their primitive activity only after a period of inactivity when the higher levels are removed.

We follow Pike in favouring the latter hypothesis. Such a view explains the increasing severity of spinal shock in the ascending vertebrate series as the long conducting paths increase in complexity. It also offers an explanation, as Pike (11, p. 151) suggests, for the decidedly less marked severity of spinal shock in young animals; for here the shifting of function forwards will not be complete and the effect of spinal section will be to isolate in the caudal segment a more active functional structure than in the adult animal. Further, it is noticeable that patients suffering from spastic paralysis do not lose the deep reflexes to the same degree as patients without any nervous lesion. Even in deep anaesthesia clonic contractions persist, and a possible explanation of this is that morphologically persistent paths have become functional subsequent to the lesion of the central nervous system. It may be supposed, therefore, that the effect of spinal shock is due to the removal of the impulses traversing descending conducting pathways which normally cross the synapse leading to the spinal motor cells. Recovery of reflex activity follows the discharge of impulses to this synapse from segmental and inter-segmental pathways which were not actively functioning in the intact animal.

III. THE FACTORS RESPONSIBLE FOR THE VARIABLE DURATION AND SEVERITY OF SPINAL SHOCK.

If spinal shock is a phenomenon wholly due to isolating the caudal segment from higher levels of activity, and the second stage of complete transverse lesions of the cord of hyperactive reflexes is due to the assumption of function of morphologically persistent pathways, the activity of which is subordinated in the intact animal, it would follow that in any given species the duration and degree of severity of spinal shock should be approximately constant. We have seen that no variable factor has so far been discovered to account for this variation except sepsis. When only non-septic cases are considered, a great variation is still found. We have seen that the degree of trauma is not responsible for these variations. Is the variability of the blood supply of the

caudal segment involved in producing variations in the period of spinal shock? It may well be that diminished blood supply produced in transection of the spinal cord, as explained in the introduction to this paper, may interfere with the rate of assumption of function and the ultimate degree of activity of the segmental and intersegmental pathways. In the present terminology, which we shall later point out should be modified, slowness in the assumption of function is said to be due to long lasting spinal shock. But it has long been known (*vide* supra) that in man and monkeys cases exist in which the reflexes fail to appear at all. In 1897 Sherrington described the permanent failure of certain spinal reflexes of the monkey as being due to "isolation dystrophy." "The withdrawal from the isolated cord of influences it is wont to receive from centres further headward may induce an alteration of trophic character in spinal cells—an 'isolation dystrophy'—visible, it may be, as Nissl's chromatolysis" (16, p. 247). But why is the exhibition of isolation dystrophy also a variable feature in the same species? This, too, may be due to a vascular disturbance. It would then become more evident in those forms in which the segmental blood supply is less regular, the complementary longitudinal anastomosis being more important in consequence. Our experimental evidence is in favour of the view that the variable degree of vascular disturbance in spinal transection in different individuals accounts for the phenomenon of isolation dystrophy engrafting itself upon the initial spinal shock due to severing aborally conducting paths in some cases and not in others.

The following experiments were carried out in order to determine the effect of cutting off the longitudinal spinal vessels to the caudal segment in transverse lesions of the medulla spinalis.

Goat (1). Operation performed 15/10/20.

With full aseptic precautions a rectangular flap of skin was reflected overlying the lower three thoracic and the upper two lumbar vertebrae. The sacrospinalis was turned laterally on each side, and the lowest three thoracic spines and the corresponding laminae were removed. This exposed the dura mater, which invests the cord closely. The dura and arachnoid were incised, and the pia mater containing the blood vessels became apparent. In the goat a postero-median artery is present. It was cauterized by the application of a heated platinum needle. One posterior nerve root and the corresponding anterior nerve root were severed on the right side. The antero-median vessel was now brought into view. This also was cauterized, together with a well-defined anastomotic vessel uniting the two median vessels on the left side. The dura mater was stitched and the layers of muscles and fasciae stitched in order.

On recovering from the anaesthetic half-an-hour after cauterizing the vessels, there was complete absence of voluntary movement of the tail and hind limbs.

These limbs were completely flaccid and the knee jerks were absent. Further examination four hours later showed a similar condition.

The following notes have been selected from reports of daily examination. The figures refer to the number of days from the day of operation, which is counted as the first day.

2nd day. There was no evidence of the functioning of organic reflexes. The somatic reflexes were absent, the flaccidity was marked. Sensations were absent behind the middle of the scar.

3rd day. Micturition was apparently automatic; the bowels opened with small result; the limbs were flaccid.

4th day. Somatic reflexes returned, with slight increase of tone in the muscles of the hind limbs. The tail was dependent. The faeces and urine were evacuated intermittently.

5th day. Bladder intermittent in action; the rectum was full of faeces presenting at the anus for some time before defaecation; the sphincter was contracted on the cessation of defaecation. The reflexes were increased. Clonus was elicited by sharply extending the hind limb on the right side.

6th day. The position attained by the limbs was not that of spasticity, either in flexion or extension, but a mid position of flexion. Clonic movement could still only be elicited on the left side; flexion reflex was present however on this side. Tapping the sacrospinalis in the lower lumbar region caused a marked contraction of the muscle.

7th and 8th days. Knee reflexes still active; clonus was less easily elicited. The limbs would stay in flexion or in extension. The reflex movements of the tail were extremely active. Clonic movements of flexion and extension of the tail were elicited.

9th day. A condition of severe diarrhoea developed and the reflex activity was somewhat depressed.

10th day–18th day. General condition improved; the signs of hyperactivity of the reflexes returned, e.g., clonus, contracting of the sacrospinalis on tapping, knee jerk.

19th–21st days. Further reduction in the hyperexcitability of the reflexes until the reflexes to the limb muscles could not be elicited. At the end of this period the urine was turbid. The animal was apparently ill.

22nd day. Constipation had been present for twenty-four hours. An enema was administered. Reflex movements in the tail were marked on stimulation in the perineal area. On distending the rectum the hind limbs were strongly flexed. Death followed.

Post mortem examination (November 5, 1920) showed unabsorbed stitches of catgut in the dura. The dural sheath contained semifluid custard-like material for the extent of 5 cm., partly above and partly below the site of operation. The cord was removed and hardened, but it was impossible to preserve it intact as the

fluid contents of the dura escaped, leaving a ragged eroded dorsal surface. Anteriorly there was a bridge of spinal cord, with the antero-median vessel upon it comparatively normal in appearance. Bacteriological examination of slides from the contents of the sheath was negative for infective organisms.

The cause of death was peritonitis. The bladder was distended with pus, and the early stage of peritonitis present was probably due to this condition.

Some of the variations in the reflexes were due to the sepsis of the bladder which supervened. But microscopical examination of the spinal cord revealed a more potent cause for the absence of reflexes in the third stage following the virtual transection of the cord by thrombosing the longitudinal blood vessels in a limited area.

As noted, there was no sign of septic meningitis, but owing to the escape of custard-like material during the process of removal the cord was extremely deficient posteriorly, where it presented a very ragged appearance. The cord was very ragged over an extent of 4 cm. Elsewhere the external surface of the cord was normal in appearance. The cut surface of the proximal end presented no abnormality. Examination of transverse sections of the distal portion, on the other hand, revealed the fact that the same custard-like material occupied the greater portion of both posterior columns of gray matter. In the further steps of preparation, these columns were almost entirely lost, two oval cavities representing their position for a distance of 10 cm. below the original lesion. Gordon Holmes (6) describes this semifluid disintegrating material as occurring for 1 cm. from the point of division at the ends of segments of the spinal cord in cases of complete transection in man. The site of operation when subjected to microscopic examination revealed large vesicular cells, which are faintly staining, containing vacuoles. Several vacuoles may be present in a single cell. The nucleus is peripheral in position, and not deeply staining. The cells of the distal segments of the cord revealed extensive chromatolysis, especially in the cells of the posterior column, which could still be seen. Dr. Oliver Latham, of Sydney, in his report on this cord, observed that some anterior column cells had escaped this degeneration. Subsequent experiments verified this interpretation.

Goat 2. Operation 9/12/21.

The technique was identical with that observed in the previous experiment, but instead of a cautery being used, a fine silk thread was passed under the pia mater to enclose the vessels at the last thoracic segment. The posterior vessel was inadvertently lifted from the surface of the cord for the extent of 6 mm., and was ruptured in the process. The distal end was securely ligatured. The anterior vessel was approached by severing two sets of nerve roots on the right side. An anastomotic vessel coursing around the right side of the cord joining the other two vessels was also ligatured at the same level.

On recovering from the anaesthetic the tail and hind limbs were completely paralyzed. The knee jerks were present. The hind limbs were somewhat extended and flexion was resisted. Four hours later the hind limbs were completely flaccid, without knee jerks. Next day the knee jerk was present on the right side only. The tail moved reflexly with defaecation. The organic functions were apparently carried out intermittently. Sensations were absent behind the level of the scar.

3rd day. There was marked spasticity in a flexed attitude, though the position was not that of extreme flexion and resistance to extension. The knee jerks were exaggerated, especially on the right side. The flexion reflex was well marked. No crossed extension of the opposite limb was observed. The animal could support itself for five seconds on the four limbs.

4th day. Marked spasticity and increased reflexes were present, with intermittent defaecation and micturition.

5th day. Spasticity, with a position of moderate flexion, was marked. The knee jerks were exaggerated, especially on the left side.

6th–15th day. Gradual diminution in the spasticity was observable. On this day the left knee jerk could not be elicited. On the right side it was not hyperactive.

16th–26th day. The knee jerk gradually diminished. On the 26th day no knee jerk was elicited on either side.

27th–39th day. The bladder and rectal functions continued to be carried on automatically.

The post-mortem examination on the 39th day (16/1/21) revealed no infection of the cord. It was slightly diminished in diameter for the extent of 3·5 cm., though normal in appearance. It was removed intact and hardened in formalin, a segment from the distal end being placed in alcohol.

The urinary bladder was normal and contracted without sign of infection. A little clear urine was found within it. The peritoneal cavity was normal. The colon was overloaded and extremely dilated. Malnutrition was apparently the cause of death.

In the absence of sepsis the reflexes disappeared in the third stage in this experiment. The cord showed identical changes to those already described, indicating that chromatolysis is responsible for interruption of the reflex arc.

To decide the relative influence of the nervous injury and vascular disturbance in the production of "isolation dystrophy" the following experiment was performed.

Goat 7. (13/6/21).

A sub-pial resection of the cord was performed in the lower thoracic region. Several segments lower the longitudinal spinal vessels were ligated. The result of this was to isolate a portion of the spinal medulla in the lower thoracic region, in

which the vascular disturbance would be relatively small. The caudal segment of the cord, on the other hand, would show the combined effect of the injury to the nervous and vascular systems, comparable to that which results from a complete transverse section of the spinal medulla. The knee jerks were elicitable thirty-five minutes after completing the second part of the operation. By the fourth day the hind limbs exhibited well-marked spasticity, e.g., clonic contractions followed stretching of the hind limb. On the thirtieth day the reflexes permanently disappeared. The animal died on the thirty-fifth day. No sepsis was present.

The gray matter of the intermediate segment of the spinal cord below the sub-pial resection revealed, on microscopic examination, numerous well-formed multipolar cells. Unfortunately, delay occurred in extracting the cord following the death of the animal, but obvious signs of chromatolysis were to be seen in the sections of the caudal segment distal to the site of ligature of the vessels, in contrast to the findings in the proximal segment.

This animal passed through a well-marked stage of spasticity prior to the onset of the third stage of absence of reflexes. It is difficult to see why such a secondary dystrophy should occur, if due simply to isolation from higher levels of nervous activity after the reflexes have been once established. It would be more likely, as is sometimes the case in man and monkeys, for the stage of so-called spinal shock to pass directly into the stage of isolation dystrophy. Once the spnial reflexes arcs have emerged from the first stage of inactivity, it would be unlikely that any gradual process of degeneration due to isolation should slowly overtake the cells concerned, rendering them inactive again. For the second stage of reflex activity may last for years in other individuals of the same species. But in both classes of cases the caudal segment has been isolated from precisely the same higher nervous levels.

On the vascular hypothesis it may well be that variations in the blood supply account for the variable time of onset and duration, and for cases in which there is entire absence of the second stage. It may be supposed in the latter instance that the blood supply of the caudal segment is so deficient that chromatolysis early sets in, and that the cells degenerate before the morphologically persistent spinal reflex arcs have time to become active. In the cases of dystrophy following heightened reflex activity, it is suggested that the blood supply of the caudal segment, though sufficient to enable the reflexes to become active, never reaches an optimum, and that a slow degeneration of the cells leads in the third stage to inactivity again. Many clinical features support this view.

The present doctrine of the causation of increased reflexes is the outcome of the views expressed by Hughlings Jackson, which have been supplemented by the interpretations of Head and his collaborators. The principle of release of function in the nervous system is well established. The result of removal of higher levels of activity is to allow positive symptoms to appear due to release

of the lower levels from control. Head (4) has recently dealt with this subject in an admirable essay. An addition to this hypothesis has been made by Head and his collaborators (notably Rivers) to explain the nature of the manifestations of release from control. They have introduced the phylogenetic factor; they seek to interpret symptoms following disease and injury of the nervous system as representing primitive activities of the nervous system at an earlier evolutionary phase. For instance, Head and Riddoch (5) say the mass reflex, consisting of withdrawal of the lower extremity from a painful stimulus, accompanied by the passage of urine, emptying of the rectum and sweating, "belongs to the ancient nonceptive mechanism" (p. 501). But Walshe (18) has pointed out such a view is difficult to reconcile with the facts of comparative physiology. "It is difficult," he says, "to see a useful protective function in a response consisting of squatting, sweating, micturating to each and every stimulus, especially when we bear in mind that the protopathic animal has no power of locating stimuli" (p. 839). The question at once arises, then, as to whether this phenomenon really is a true expression of the activity of co-ordinated reflex arcs within the caudal segment of the transected cord. Head and Riddoch (5, p. 501) point out that when primary extensor activity reappears in the distal end of the spinal cord, flexion becomes controlled and modified as its direct co-relative in postural tone. To establish the mass reflex as a normal expression of the isolated segment of the cord it was necessary for these authors to show that "so long as local reflex signature is not destroyed, and so long as primary postural reflexes can be obtained, the lower end of the spinal cord has not been liberated from control of the parts above the lesion." But they have failed to establish their case that primary extensor manifestations indicate an incompletely divided cord, for some cases of complete spinal transection exhibit extensor responses and not flexor only, as is shown by Marie and Foix (9). Head and Riddoch (5, p. 505), in quoting these results, say that "evidently Marie and Foix were dealing with a different group of cases from those on which we have based the main argument in this paper." But Rennie (12) has described the course of the symptomatology of one of Head and Riddoch's patients who, at operation, before returning to this country, was proved to have a completely severed spinal cord, the gap between the two segments being 2·5 cm. In this patient extensor spasms were of frequent occurrence, and Rennie concludes that though there is a preponderance of tone and reflex activity in the flexors in complete transverse spinal cases, these features are not entirely confined to this group. Extra-pyramidal pathways are responsible, on the other hand, when intact, for the transference of the preponderance of tone and reflex activity to the extensors. The flexed posture is, therefore, characteristic of the spinal animal which is reminiscent of the squatting attitude of the frog. The extended posture characterises the decerebrate preparation the animal so exhibiting "reflex standing," indicating the normal posture of higher forms. But extensor activity is

still possible in the isolated spinal cord, and this manifestation is not an absolutely reliable index that the cord has been incompletely and not completely divided (cf. Riddoch, 13). It can now be recognized that skeletal muscle tone is constituted by two elements which, following Langelaan (8), we have referred to in previous papers (14, 7) as contractile tone, maintained by a somatic proprioceptive arc, and plastic tone, maintained by a sympathetic proprioceptive arc. The former, acting differentially, imposes a posture. The latter maintains this posture. The reason that rigidity is not seen in spinal animals to the same extent as in the decerebrate condition is that a prespinal centre is involved in the proprioceptive reflex arc, subserving plastic tonus. In an ascending vertebrate series the spinal sympathetic reflex arc becomes more and more subservient to the longer prespinal sympathetic reflex, as we have already shown (7). This explains why the spinal dog is unstable in the standing attitude. The postural mechanism is therefore a double one. Two nervous pathways subserve the two elements of tone responsible for the assumption and maintenance of posture respectively. Extensor spasms will therefore only be transient when present in spinal man, but that they do occur is undeniable.

We are now faced with another aspect of the variation in symptoms following complete transection of the cord. Those cases in which reflexes reappear may be grouped into two classes, viz., those with hyperexcitability accompanied by local sign and extensor responses, and those exhibiting the mass reflex. The first condition suggests the co-ordinated effects of normal reflex activity described by Sherrington (15). Speaking of stimulating the apparent pathways of the isolated segment, he shows that strong stimuli lead to a widespread harmonious effect. Progressively weaker stimuli lead to "the non-appearance of the reaction in a progressively increasing number of the harmoniously acting regions of the musculature."

The mass reflex, on the other hand, is "a diffuse outburst of motor energy" (5, p. 494), in which even the accompanying micturition is at least in part due to contraction of the recti abdominus. Is this, as these authors claim, a sign that lower mechanisms are set free from control? The hyperexcitability in the first group of cases is undoubtedly to be accounted for by release from the control of higher centres due to the complete transection. Is release of a greater degree to be seen in other individuals of the same species, following the same injury? Obviously, this explanation is inadequate. The mass reflex, in so far as it is a diffuse outburst of motor activity, is due, not to release, but hyperexcitability of the cells. Unlike the harmonious action of the spinal animal already described, "there is a simultaneous discharge of reflex action which are in no sense allied, but comprise all the reactions of which the cord is capable" (18, p. 839). The co-ordinated reflex mechanism is obviously impaired.

Just as our experiments were completed, and we had come to the view that vascular disturbance of varying degree was responsible for the variations in the

degree and nature of the reflex activity of the isolated spinal cord, Walshe's paper (18) appeared on this subject. He assembled the physiological evidence at present available to show that the mass reflex resembled the activities of the spinal cord when deprived of an adequate oxygen supply (Kaya and Starling), or when minimal degrees of CO_2 excess (Mathison) are present. Walshe also cites Trotter's view that cyanosis of the cerebral cortex is associated with true excitability of nerve cells accounting for the so-called irritative symptoms in this condition. We may add that Stewart and Pike (17) have shown that restoration of the cerebral circulation following short periods of anaemia due to clamping the head arteries is accompanied by convulsive seizures. Even, for example, auditory stimuli excite such seizures. We therefore suggest, with Walshe, that chronic anoxamia accounts for the mass reflex, and that the failure of restoration of the optimum blood supply after transection in such cases leads to chromatolysis of the nerve cells and eventually the reflexes fail. When the caudal segment is adequately supplied with blood the spinal reflex arcs may bring about co-ordinated reflex acts, as in the other group of cases. And, finally, that a totally inadequate supply of blood will lead directly to "isolation dystrophy" before the assumption of function by the reflex spinal arcs has occurred. These variations are incapable of explanation on the hypothesis of release of function alone. There may be combined with release of function vascular changes leading to rapid degeneration, or a temporary stage of hyper-excitability of the nerve cells.

In an article on the "Spinal Cord," Sherrington (15) has an admirable section on the vascular conditions influencing the condition of the central organ. We wish to emphasize the conclusion that vascular disturbance plays its part, as truly as section of the conducting pathways, in the production of symptoms following complete transection of the spinal cord.

IV. TERMINOLOGY.

A review of the experimental evidence and its discussion in the present paper reveals how unsatisfactory our terminology at present is. We have seen that it is the process of isolation of morphologically persistent pathways, relatively inactive in the intact animal that causes so-called spinal shock, for time is demanded for their full assumption of function. "Isolation inactivity" would be a preferable term for spinal shock. "Isolation release" and "isolation hyper-excitability" would be suitable and readily understood terms to describe the double effect of nervous and vascular disturbances respectively following a transection of the cord; and Sherrington's term "isolation dystrophy" may well be retained for the end result of the nutritional changes induced by interference with the blood supply of the isolated caudal segment.

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V. SUMMARY OF EXPERIMENTS.

Macacus rhesus 1. Sup-pial resection. On the twenty-first day, when spasticity was well marked, the pial bridge and its contained blood vessels were removed.

Macacus rhesus 2. Saline block of the long conducting pathways was effected temporarily. Later, absolute alcohol was injected into the cord without damaging the pia mater.

Macacus rhesus 3. A permanent transection without damaging the pia was produced by absolute alcohol.

Goat 1. The longitudinal spinal blood vessels were cauterized and the animal studied throughout the three stages following complete transverse lesion of the spinal cord for twenty-two days.

Goat 2. The longitudinal spinal blood vessels were ligatured and the animal studied throughout the three stages following complete transverse lesion of the spinal cord for thirty-nine days.

Goat 3. Control experiment in which all the trauma produced in the former two experiments was reproduced without actually ligaturing the vessels.

Goat 4. Five days after sub-pial resection the empty pial bridge was severed.

Goat 5. The same experiment as for Goat 4, the interval between the two operations being 2 hours 40 minutes.

Goat 6. The same experiment as for Goat 4, the interval between the two operations being ten minutes.

Goat 7. A sub-pial resection of the cord was performed in the lower thoracic region and the longitudinal spinal vessels were ligatured in the upper lumbar region below this.

Dog 1. The same experiment as for Goat 4, the interval between the two operations being two minutes.

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